



Product Information

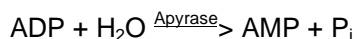
SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of APYRASE

(EC 3.6.1.5)

ADP as Substrate

PRINCIPLE:



Abbreviations:

ADP = Adenosine 5'-Diphosphate

AMP = Adenosine 5'-Monophosphate

P_i = Inorganic Phosphate

CONDITIONS: T = 30°C, pH 6.5, A_{660nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 40 mM Succinate Buffer with 4 mM Calcium Chloride, pH 6.6 at 30°C
(Prepare 100 ml in deionized water using Succinic Acid, Free Acid, Sigma Prod. No. S-7501, and Calcium Chloride, Dihydrate, Sigma Prod. No. C-3881. Adjust to pH 6.6 at 30°C with 5 M NaOH.)
- B. 2.0 mM Adenosine 5'-Diphosphate Solution (ADP)
(Prepare 15 ml in Reagent A using Adenosine 5'-Diphosphate, Di(Monocyclohexylammonium) Salt, Sigma Prod. No. A-4386. Adjust to pH 6.5 at 30°C using 1 M NaOH.)
- C. Apyrase Enzyme Solution
(Immediately before use, prepare a solution containing 0.5 - 1.5 ADPase units/ml of Apyrase in cold deionized water.)
- D. Phosphorus Standard Solution
(Use Phosphorus Standard Solution, Sigma Prod. No. 661-9. The concentration is 20 µg/ml, 0.645 µmoles/ml.)
- E. 10% (w/v) Ammonium Molybdate Solution (Amm. Moly.)
(Prepare 25 ml in 10 N H₂SO₄ using Molybdic Acid, Ammonium Tetrahydrate Salt, Sigma Prod. No. M-0878.)

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REAGENTS: (continued)

- F. Taussky-Shorr Reagent (TSCR)
(Prepare by adding 10 ml of Reagent E to 70 ml of deionized water. Then add a 5 g vial of Ferrous Sulfate, Heptahydrate, Sigma Prod. No. F-0131, and mix until dissolved. Add enough deionized water to a final volume of 100 ml.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable containers:

	<u>Test</u>	<u>Blank</u>
Reagent B (ADP)	1.90	1.90

Equilibrate to 30°C. Then add:

Deionized Water	-----	0.10
Reagent C (Enzyme Solution)	0.10	-----

Immediately mix by swirling and incubate at 30°C for exactly 10 minutes. Then add:

Reagent F (TSCR)	5.00	5.00
Deionized Water	3.00	3.00

Mix by swirling and immediately (within 1-2 minutes) transfer to suitable cuvettes and record the A_{660nm} for both Test and Blank using a suitable spectrophotometer.

COLORIMETRIC ASSAY:

Standard Curve:

Prepare a standard curve by pipetting (in milliliters) the following reagents into suitable containers:

	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Std 4</u>	<u>Std 5</u>	<u>Std Blank</u>
Deionized Water	4.50	4.00	3.50	3.00	2.50	5.00
Reagent D (Phosphorus Std)	0.50	1.00	1.50	2.00	2.50	0.00
Reagent F (TSCR)	5.00	5.00	5.00	5.00	5.00	5.00

Mix by swirling and immediately (within 1 - 2 minutes) transfer to suitable cuvettes and record the A_{660nm} for Standards and Blank.

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CALCULATIONS:

Standard Curve:

$$\Delta A_{660\text{nm}} \text{ Standard} = A_{660\text{nm}} \text{ Standard} - A_{660\text{nm}} \text{ Standard Blank}$$

Prepare a standard curve by plotting $\Delta A_{660\text{nm}}$ Standard versus μmoles of Phosphate.

Sample Determination:

$$\Delta A_{660\text{nm}} \text{ Test} = A_{660\text{nm}} \text{ Test} - A_{660\text{nm}} \text{ Blank}$$

Determine the micromoles of phosphate liberated using the Standard curve.

$$\text{Units/ml enzyme} = \frac{\mu\text{moles of Phosphate released})(\text{df})}{(10)(0.1)}$$

df = Dilution factor

10 = Time (in minutes) of assay as per the Unit Definition

0.1 = Volume (in milliliter) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will liberate 1.0 μmole of inorganic phosphate from adenosine 5'-diphosphate per minute at pH 6.5 at 30°C.

FINAL ASSAY CONCENTRATION:

In a 2.00 ml reaction mix, the final concentrations are 38 mM succinate, 3.8 mM calcium chloride, 1.9 mM adenosine 5'-diphosphate, and 0.05 - 0.15 unit apyrase.

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REFERENCES:

Tausky, H.H. and Shorr, E. (1953) *Journal of Biological Chemistry* **202**, 675-685

Traverso-Cori, A., Chaimovich, H., and Cori, O. (1965) *Archives of Biochemistry and Biophysics* **109**, 173-184

NOTES:

1. This assay is based on the cited references.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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